

CLAIMS

1. A method for determining a site of ubiquitination comprising:

obtaining a plurality of ubiquitinated polypeptides;
digesting the ubiquitinated polypeptides with a protease, thereby
generating a plurality of test peptides;
determining the presence of an isopeptide bond in a test peptide by
mass spectrometry, wherein the presence of the bond indicates a site of
ubiquitination.
2. A method for determining a site of ubiquitination comprising:

obtaining a plurality of ubiquitinated polypeptides;
digesting the ubiquitinated polypeptides with a protease, thereby
generating a plurality of test peptides, at least some of which comprise a
ubiquitin remnant;
identifying a mass difference between a test peptide and a reference
peptide comprising a known identical amino acid sequence as the test peptide,
the mass difference corresponding to the mass of the ubiquitin remnant,
wherein detection of the mass difference indicates a site of
ubiquitination in the test peptide.
3. The method according to claim 1 or 2, further comprising ionizing a
test peptide.
4. The method according to claim 3, further comprising fragmenting the
ionized test peptide.
5. The method according to claim 1 or 2, further comprising mapping a
sequence of a test peptide comprising a ubiquitin remnant to a polypeptide sequence
comprising the same amino acid sequence as the test peptide.
6. The method according to claim 3, wherein ionizing is performed by an
electrospray.
7. The method according to claim 1, wherein ubiquitinated polypeptides
are obtained by contacting cellular polypeptides with binding partners which bind to a

ubiquitin molecule thereby forming ubiquitinated polypeptide:binding partner complexes; and isolating the complexes.

8. The method according to claim 1, wherein the step of isolating comprises separating the ubiquitinated peptides.

9. The method according to claim 8, wherein separating is performed by at least one round of liquid chromatography.

10. The method according to claim 9, wherein chromatography is performed by reversed-phase liquid chromatography or by HPLC.

11. The method according to claim 1 or 2, wherein the ubiquitin remnant comprises Gly-Gly amino acid residues.

12. The method according to claim 1 or 2, further comprising detecting multiple ubiquitination sites in a single polypeptide.

13. The method according to claim 12, further comprising determining the relative abundance of ubiquitination at one or more of the multiple sites in a plurality of polypeptides.

14. The method according to claim 7, wherein the binding partners specifically bind to a tag molecule linked to ubiquitin.

15. The method according to claim 14, wherein the ubiquitin molecule comprises histidine-tagged ubiquitin.

16. The method according to claim 14, wherein the ubiquitinated polypeptides are obtained from a first cell expressing a tagged ubiquitin molecule.

17. The method according to claim 16, wherein the first cell is a mammalian cell.

18. The method according to claim 17, wherein the first cell is a mouse cell.

19. The method according to claim 1 or 2, further comprising identifying ubiquitination sites for a plurality of polypeptides in a first cell.

20. The method according to claim 19, further comprising identifying ubiquitination sites for a plurality of cellular polypeptides in a second cell.
21. The method according to claim 20, further comprising comparing ubiquitination sites identified in the first cell to the sites identified in the second cell.
22. The method according to claim 20, wherein the first cell is a normal cell and the second cell is from a patient with a pathological condition.
23. The method according to claim 22, wherein the pathological condition is a neurodegenerative disease.
24. The method according to claim 20, wherein the second cell differs from the first cell in expressing a recombinant DNA molecule.
25. The method according to claim 19, further comprising contacting the first cell with a compound and comparing ubiquitination sites identified in the first cell with ubiquitination sites in a second cell not contacted with the compound.
26. The method according to claim 19, further comprising generating a database comprising data files storing information relating to ubiquitination sites for a plurality of polypeptides for a plurality of different cells.
27. The method according to claim 2, wherein the mass difference is about 114 daltons.
28. The method according to claim 1 or 2, wherein the site of ubiquitination is correlated with disease and detection of ubiquitination at the site is associated with risk of the disease.
29. The method according to claim 1 or 2, further comprising the step of determining the presence, site, or amount of a protein modification other than ubiquitination.
30. A computer memory comprising data files storing information relating to ubiquitination sites for a plurality of polypeptides for a plurality of different cells.

31. A kit comprising a ubiquitin binding molecule and one or more components selected from the group consisting of: a protease; an isotope-coded affinity tag; a pair of isotope-coded affinity tags; an affinity tag coupleable to an isotope; an isotope-labeled peptide comprising Gly-Gly residues, a peptide comprising Gly-Gly residues coupleable to an isotope; an isotope-labeled Gly-Gly dipeptide; a Gly-Gly dipeptide coupleable to an isotope; a mass modifying moiety; a sample plate for use with a mass spectrometer; a light-absorbent matrix; software for analyzing mass spectra; and access to a computer memory comprising information relating to ubiquitination sites for a plurality of polypeptides for a plurality of different cells.

32. A kit comprising an antibody that specifically recognizes a peptide product of a protease-digested ubiquitinated protein which comprises a ubiquitin remnant.

33. The kit according to claim 32, wherein the peptide comprises a lysine residue at position 6, 11, 27, 29, 33, 48, and 63 of the ubiquitin polypeptide.

34. A kit comprising an antibody which specifically recognizes a ubiquitin polypeptide ubiquitinated at one or more of the K⁶, K¹¹, K²⁷, K²⁹, K³³, K⁴⁸, and K⁶³ sites.

35. A kit according to claim 32 or 33, further comprising an antibody which specifically recognizes a phosphorylated form of the peptide.

36. A kit according to claim 34, wherein the kit further comprises an antibody which recognizes a phosphorylated form of the polypeptide.

37. The kit according to claim 35, wherein the antibody recognizes a phosphate group at Ser⁵⁷.

38. A method for detecting a site and/or amount of ubiquitination in a ubiquitin molecule, comprising:

detecting a ubiquitin remnant in a peptide product of a digested ubiquitin polypeptide, wherein the peptide comprises a lysine residue at position 6, 11, 27, 29, 33, 48, and 63 of the ubiquitin polypeptide.

39. The method according to claim 38, wherein the presence of a ubiquitin remnant at one or more of the sites is correlated with the presence or absence of a pathology.

40. The method according to claim 38, further comprising determining the presence or absence of a phosphate group on the peptide.

41. A method for detecting a site and/or amount of ubiquitination in a ubiquitin polypeptide, comprising:

detecting a ubiquitin molecule at one or more of more lysines at residues 6, 11, and 27 of the ubiquitin polypeptide.

42. An antibody specific for a modified form of a ubiquitin molecule which does not recognize a non-modified form of the molecule, wherein the modified form of the ubiquitin molecule is ubiquitinated at one or more of K⁶, K¹¹, K²⁷, K²⁹, K³³, K⁴⁸ and K⁶³ sites.

43. An antibody specific for a modified form of a ubiquitin molecule which does not recognize a non-modified form of the molecule, wherein the modified form of the ubiquitin molecule is phosphorylated at Ser⁵⁷.

44. A composition comprising a peptide internal standard comprising a peptide labeled at a ubiquitination site.